Effects of diet on rat intestinal soluble hexokinase and fructokinase activities

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WEISER, MILTON M., HELEN QUILL, AND KURT J. ISSELBACHER. Effects of diet on rat intestinal soluble hexokinase and fructokinase activities. Am. J. Physiol. 221(3): 844–849. 1971.—The effects of diet on rat intestinal hexokinase activity, hexokinase isozymes, and fructokinase activity were evaluated by separation of these enzymes with DEAE-cellulose chromatography. Casein noncarbohydrate diets decreased intestinal hexokinase activity when compared to starved animals. High-fructose diets did not increase hexokinase activity. High-glucose or high-sucrose diets increased hexokinase activity but only as compared to the casein-fed animals. A major effect of high-glucose diets was a relative increase in jejunal type II hexokinase over type I. The increased ratio of type II over type I hexokinase appeared to be confined to the upper absorbing cells of the villus, suggesting a noninductive effect of high-glucose concentrations. Type IV (glucokinase) was never detected. Hexokinase activity was consistently higher in the ileum while fructokinase activity was higher in the jejunum. Starved animals and casein noncarbohydrate-fed animals had equally low fructokinase activity. All three high-carbohydrate diets (glucose, fructose, and sucrose) increased fructokinase activity, though the increase was higher on the high-fructose and high-sucrose diets.

hexokinase isozymes; glucokinase; DEAE-chromatography

THERE ARE CONFLICTING REPORTS in the literature on the response of intestinal mucosal glycolytic enzymes to diet. Stifel et al. (16) reported that intestinal hexokinase, glucokinase, and fructokinase specific activities were increased in animals fed any diet and showed highest activities in animals fed carbohydrate-rich diets. They termed this an "adaptive" response and recently Rosensweig, Herman, and Stifel (13) suggested a new human clinical entity in which diarrhea was believed secondary to failure of the adaptive response to increase intestinal hexokinase and glucokinase activities. Srivastava et al. (15) initially also suggested that fed animals showed a higher specific activity for intestinal hexokinase. However, in later work they proposed that the response to diet was due to an intracellular shift from particulate to soluble hexokinase secondary to a high-glucose concentration in the epithelial cell rather than any fundamental change in enzyme synthesis (10). These workers, in contrast to Stifel et al. (16), could not detect any glucokinase, i.e., the high-Kₐ hexokinase or type IV isozyme of Kaizen and Schimke (7).

Except for the studies by Srivastava et al. (15), the responses of these intestinal glycolytic enzymes to diet were evaluated without separation of the hexokinase isozymes and without separation of specific fructokinase from nonspecific hexokinases. This latter separation is important since both fructokinase and hexokinase phosphorylate fructose, although the respective products are different. Furthermore, the usual fructokinase assays do not distinguish between fructokinase and hexokinase activity (18, 19). Separation of the hexokinase isozymes is important since they have some tissue specificity and respond differently to diet. In the liver, glucokinase is affected by diet while in other tissues, hexokinase isozyme type II appears to respond to dietary changes (3, 6–8, 13).

Recently, we separated specific fructokinase from intestinal hexokinase isozymes types I and II by DEAE-chromatography (19). No significant glucokinase (type IV) activity was detected in either rat or guinea pig mucosa. It was also shown that hexokinase contributes significantly to the total fructose phosphorylating activity of the small intestine, a fact which makes separation of fructokinase from hexokinase essential in evaluating dietary effects. To overcome this latter problem, we developed a modified assay for fructokinase, in which the hexokinase activity is eliminated, and which is therefore applicable to crude tissue preparations (18).

The development of these techniques provided an opportunity to define more accurately the effects of diet on these enzymes. It was found that there is indeed a response of intestinal hexokinase and fructokinase to diet and that these responses are specific for a given dietary sugar. However, the data indicate that there is no adaptive response for intestinal hexokinase.

MATERIALS AND METHODS

Enzyme assays. Hexokinase was determined spectrophotometrically by the method of Sharma et al. (14). A radioactive assay for hexokinase and fructokinase was also used as previously described (19), primarily for estimating column fractions. However, in crude tissue preparations a new assay for fructokinase was used (18) in which the pH of the enzyme preparation is first brought to 6.0, readjusted after centrifugation to pH 7.4, and the activity then assayed in the presence of 50 mm N-acetylglucosamine. These procedures effectively excluded hexokinase activity leaving fructokinase activity intact (18). Sucrase and maltase were determined according to the methods of Messer and Dahl.

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DIET EFFECT ON INTESTINAL HEXOKINASE, FRUCTOKINASE

TABLE 1. Effect of diet on rat intestine hexokinase specific activity

<table>
<thead>
<tr>
<th>Diet</th>
<th>Entire Small Intestine</th>
<th>Jejunum</th>
<th>Ileum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starved 3 days</td>
<td>12.6 ± 1.38 (10)</td>
<td>11.8 ± 0.37 (23)</td>
<td>18.7 ± 1.11 (12)</td>
</tr>
<tr>
<td>Cеасин (non-CHO)</td>
<td>9.4 ± 0.82 (3)</td>
<td>9.7 ± 0.33* (39)</td>
<td>15.3 ± 0.80 (16)</td>
</tr>
<tr>
<td>High glucose</td>
<td>13.5 ± 1.43 (3)</td>
<td>12.3 ± 0.75† (14)</td>
<td>16.2 ± 1.08 (13)</td>
</tr>
<tr>
<td>High fructose</td>
<td>ND</td>
<td>9.3 ± 0.64† (8)</td>
<td>16.1 ± 1.80 (5)</td>
</tr>
</tbody>
</table>

Values are mean hexokinase specific activities in nanomoles per minute per milligram protein followed by SEM. The numbers of animals tested are in parentheses. ND = not done. The following effects of diet were compared and found to be significantly different: starved vs. casein, P < 0.001; casein vs. high glucose, P < 0.001; starved vs. high fructose, P < 0.001; high glucose vs. high fructose, P < 0.005. Jejunum differs from ileum in all diets, P < 0.001. The following jejunal values were compared and found not to differ significantly: starved vs. high glucose, and casein vs. high fructose. Ileal values were found not to differ significantly except for starved vs. casein, P < 0.25. *Three to four days on diet, 0.5 ± 0.41; 6-7 days on diet, 9.7 ± 0.37. †Three to four days on diet, 11.6 ± 0.57; 6-7 days on diet, 12.4 ± 1.60. ‡Three to four days on diet, 10.0 ± 2.03; 6-7 days on diet, 8.6 ± 0.40.

RESULTS

In evaluating the effects of diet on hexokinase and fructokinase activity, animals were treated as described by Stifel et al. (16). All animals were fasted for 3 days and either sacrificed or placed on special diets for 3 days and then sacrificed. Some animals were kept on special diets over 3 days, but they did not appear to show any significant differences from those sacrificed at 3 days (see footnotes, Table 1). Only soluble hexokinase activity was evaluated, although 50% of the crude homogenate hexokinase activity is particulate (19). The function of the particulate hexokinase is not clear and has been considered not to participate in glycolysis (10). In evaluating hexokinase, the spectrophotometric assay of Sharma et al. (14) was used. Figure 1 summarizes the assay and the linearity of the reaction. Operationally, the difference in rate between the cuvette containing 100 mM glucose and that with 0.5 mM glucose has been considered an estimate of glucokinase (i.e., type II hexokinase) in those tissues known to contain significant glucokinase activity. In mammalian tissues significant amounts of this activity have been detected only in liver. Small increases in activity with 100 mM glucose can be attributed to type II hexokinase (6). As previously reported (19), there is no significant glucokinase activity in the intestine, nor were we able to detect the emergence of glucokinase activity in those animals on special carbohydrate diets either by assay of crude preparations or by isolation on DEAE-cellulose column chromatography.

Dietary effects on intestinal hexokinase. The data in Table 1 indicate the effects of starvation and three special diets on the levels of soluble hexokinase activity in rat intestine. Animals fed a high-casein, noncarbohydrate diet showed a significant decrease in hexokinase activity compared to fasting. No statistically significant increase in intestinal hexokinase activity was found in animals fed a high-glucose diet when compared to starved rats. However, there was a highly significant increase in intestinal hexokinase activity of animals on a high-fructose diet when compared to those on the casein noncarbohydrate diet. This hexokinase response to dietary glucose was primarily in the jejunum and was apparently quite specific in that a high-fructose diet did not produce an increased hexokinase activity. The hexokinase activity in the ileum was significantly higher than in the jejunum in all animals, irrespective of diet, and was highest...
in the ileum of starved rats. The ileum did not appear to respond to high-carbohydrate diets, but in rats fed a high-casein diet there was a significant decrease in hexokinase activity compared to starved animals. The unusual distribution of hexokinase is illustrated in Fig. 2, which shows that both specific hexokinase activity and total hexokinase activity steadily increased toward the distal small intestine.

In another series of animals the effects of a high-sucrose diet on hexokinases and disaccharidases were compared (Table 2). The increased response of sucrase and maltase to diet reported by other laboratories (2, 5) was confirmed. However, a casein diet appeared to depress sucrase activity when compared with starved animals. A high-sucrose diet increased the hexokinase activity to the same level as occurred with the high-glucose diet and was statistically significant when compared with the casein, noncarbohydrate-fed animals. Thus, the response of intestinal hexokinase to diet appears to be specific for glucose, or glucose-containing disaccharides.

At least four isozymes of hexokinase are known to exist, and the distribution of these isozymes is partly tissue specific (7). Diet or exposure to substrate appears to affect the type II isozyme except in the liver where only type IV responds to diet (6, 14). In order to evaluate the effect of diet on the molecular species of intestinal hexokinase, we first demonstrated the presence in significant amounts of only two intestinal hexokinases, types I and II (19). The effect of diet on hexokinase isozymes was evaluated using these isolation techniques. The 105,000 X g supernatant of rat intestinal epithelial homogenates was placed on DEAE-cellulose columns and the activity eluted as previously described (19). Figure 3 shows the typical elution pattern. DEAE-cellulose chromatography not only separated the hexokinase types I and II, but also separated specific fructokinase from the fructose phosphorylating activity of the hexokinases.

The effects of diet on hexokinase isozymes are shown in Table 3. The results are expressed in terms of the percent contribution of type I and type II to the total eluted hexokinase activity. Only in the animals fed a high-glucose diet was there a significant and reproducible change with a decrease in type I relative to an increase in type II, a change reflected in the decreased ratio from 0.45 to 0.64 in the nonglucose-fed animals to 0.28 in the glucose-fed animals.

It remained to be determined whether this change in the ratio of hexokinase isozymes on a high-glucose diet involved all the cells of the villus including the crypts or only a select group of cells most directly involved with the absorption of glucose. This possibility was evaluated by comparing the DEAE-cellulose chromatographic patterns of hexokinase isozymes from superficial and deep mucosal scrapings (Fig. 4). It appeared that the main shift to an increased dominance of type II occurred in the superficial scrapings. This suggests that the increase in hexokinase activity on a high-glucose diet was primarily an increase in type II hexokinase and was confined to those cells in the jejunum most exposed to the high-luminal glucose concentrations.

**Dietary effects on intestinal fructokinase.** In contrast to intestinal hexokinase, fructokinase appears to be localized predominantly in the proximal portions of the rat intestine (Fig. 2). The response of jejunal fructokinase to diet was evaluated in crude enzyme preparations with a new assay.

**TABLE 2. Effect of diet on rat jejunal enzymes**

<table>
<thead>
<tr>
<th>Diet</th>
<th>Hexokinase</th>
<th>Sucrase</th>
<th>Maltase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starved 3 days</td>
<td>11.8 ± 0.37</td>
<td>6.3 ± 0.09</td>
<td>0.28 ± 0.033</td>
</tr>
<tr>
<td>Casein (non-CHO)</td>
<td>9.7 ± 0.33</td>
<td>0.08 ± 0.004</td>
<td>0.29 ± 0.013</td>
</tr>
<tr>
<td>High sucrose</td>
<td>14.0 ± 0.66</td>
<td>0.18 ± 0.017</td>
<td>0.50 ± 0.033</td>
</tr>
</tbody>
</table>

Values are mean specific activities in nanomoles per minute per milligram protein followed by the SEM. The numbers of animals tested are in parentheses. Statistics for hexokinase: starved vs. high-sucrose diet, P < .0025; casein vs. high sucrose, P < .001.
which eliminates hexokinase contributions to fructose phosphorylation (18). The data obtained are given in Table 4 and indicate a fairly specific response to fructose. In contrast to intestinal hexokinase, fructokinase was not depressed by a high-casein noncarbohydrate diet. A high-glucose diet appeared to increase fructokinase activity but not to the extent seen with a high-fructose or high-sucrose diet. That this response of fructokinase to a high-fructose diet was specific for fructokinase was also demonstrated by data obtained with the DEAE-column chromatography (Table 5). By determining the percent contribution of isolated fructokinase and hexokinase to the total fructose phosphorylating activity eluted from the column (see Fig. 3), it was found that hexokinase accounted for 60 70% of the total activity when the animal was starved, or was fed a high-casein or high-glucose diet (Table 5). Only a high-fructose diet reversed this to the point where fructokinase accounted for 66% of the total fructose phosphorylating activity. This reversal may be explained by the data showing that a high-fructose diet increased jejunal fructokinase activity without increasing hexokinase activity (Table 1).

DISCUSSION

One of the primary problems in explaining any dietary effect on mammalian tissue hexokinase activity is the fact that most cells are continuously in contact with a fluid having a relatively constant glucose concentration and also that most cells are capable of gluconeogenesis, especially the liver cell. Sharma et al. (14) explain the hepatic dietary response by indicating that only glucokinase appeared to be affected by dietary glucose. Evidence suggests that this effect may be controlled by insulin (14). Brown et al. (3) confirmed the response of liver glucokinase to the nutritional state in both dog and man. They used the starch-gel electrophoretic separation of hexokinases developed by Katzen et al. (7) and were able to show that this glucokinase was the type IV hexokinase isozyme (equivalent to the high-$K_m$ hexokinase). Sharma et al. (14) did not find any effect of diet on liver low-$K_m$ hexokinase activity. In other tissues, however, type II hexokinase was shown to respond to various physiological factors, such as substrate concentration, starvation, diabetes, and diet (6, 8).

It was thus of considerable interest when Stifel et al. (16) reported that intestinal hexokinase responded to a number of different diets, including a noncarbohydrate diet, with variable increases in enzyme activity over that found in starved animals. Furthermore, they described the presence of an intestinal glucokinase which similarly responded to these diets. However, differentiation between hexokinase activity and glucokinase activity was based solely on the reactions obtained with two different concentrations of glucosuc using a crude tissue extract. No other investigators

<table>
<thead>
<tr>
<th>Diet</th>
<th>Fructokinase</th>
</tr>
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<tbody>
<tr>
<td>Starved 3 days</td>
<td>9.4 $ \pm $ 0.55 (6)</td>
</tr>
<tr>
<td>Casein (non-CHO)</td>
<td>10.0 $ \pm $ 0.48 (25)</td>
</tr>
<tr>
<td>High glucose</td>
<td>12.6 $ \pm $ 0.58 (6)</td>
</tr>
<tr>
<td>High fructose</td>
<td>16.3 $ \pm $ 1.97 (8)</td>
</tr>
<tr>
<td>High sucrose</td>
<td>16.7 $ \pm $ 1.40 (11)</td>
</tr>
</tbody>
</table>

Values are mean fructokinase specific activities in nanomoles per minute per milligram protein followed by the SEM. The numbers of animals are in parentheses. The following effects of diet were found to differ significantly: starved or casein vs. high glucose, $P < .025$; starved or casein vs. high fructose or high sucrose, $P < .001$. The following effects were found not to differ significantly: starved or casein, or any of the high-carbohydrate diets compared to each other.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Total Fructose Phosphorylating Activity</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>From Fructokinase</td>
<td>From Hexokinase</td>
</tr>
<tr>
<td>Starved 3 days</td>
<td>29</td>
<td>71</td>
</tr>
<tr>
<td>Casein (non-CHO)</td>
<td>39</td>
<td>61</td>
</tr>
<tr>
<td>High glucose</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td>High fructose</td>
<td>66</td>
<td>34</td>
</tr>
</tbody>
</table>

The values type I/type II are in percent of total activity eluted from the column.
have reported the presence of glucokinase in rat small intestine based either on starch gel electrophoresis or DEAE-chromatography (7, 8, 15). A recent report from this laboratory also indicated that there was no significant glucokinase in rat small intestine (19). In that study careful attempts were made to detect the presence of glucokinase, a relatively stable isozyme. Extremely small amounts of an activity which had kinetic properties suggestive of glucokinase were detected, but there was no evidence that this corresponded to diet either by assay on crude preparation or by DEAE-chromatography.

However, in the present studies a response of rat intestinal hexokinase to diet was partially confirmed. It was different from that reported by Stiefel et al. (16) in that it appeared to be more specific with regard to the dietary sugar and the response was of much smaller magnitude. Stiefel et al. (16) showed a fourfold increase in rat jejunal hexokinase over fasting controls in animals given a high-glucose diet, and all diets were reported to produce increased hexokinase activity (although the high-glucose diet showed the largest increase).

As shown in Tables 1 and 2, the response of jejunal hexokinase appeared to be specific for glucose and sucrose diets. The maximum increase was approximately 25-40% compared to a noncarbohydrate diet, and this increase was not significant compared to the fasting state. Since normal laboratory rat chow contains high concentrations of sucrose, our findings with intestine are comparable to those reported with rat liver (14) and adipose tissue (6). Those reports showed little change in low-K, hexokinase activity between fasting and normally fed animals. Our findings are also compatible with the data of Potter et al. (12) who showed that in rats adapted to 36-hr fasting periods, liver hexokinase differed from other carbohydrate-metabolizing enzymes in its cyclic variation; at the end of a 36-hr fast, hexokinase activity was at the level of normally fed animals.

In order to define further the effect of dietary glucose on intestinal hexokinase, changes in individual intestinal hexokinase isozymes were investigated (Fig. 3 and Table 3). A relative increase of type II hexokinase over type I was found. These results confirm those of Srivastava et al. (15). However, this change was most apparent in the cells occupying the tip of the villus (Fig. 4). This finding appears to support the contention of Mayer et al. (10) that the effect of the high-glucose diet is due to the local concentration of the sugar which may alter the reactivity of the enzyme or its cellular compartmentalization. The changes observed with glucose infusion by Mayer et al. (10) were quite rapid and indicated that they were not due to a true adaptive response, i.e., synthesis of new enzyme. Another explanation for our findings could be that the increase in hexokinase type II was related to increased glucose-transport demands. However, no evidence exists that glucose is phosphorylated in transport across the intestine (17). Other possibilities are that the increased glucose concentration inhibits a repressor, alters an endogenous hexokinase inhibitor, or simply stabilizes the enzyme.

An additional feature of intestinal hexokinase is its distribution along the small intestine. As shown in Fig. 2 and in Table 1, the distal small intestine always had higher hexokinase activity, both overall and specific activity. This was in marked contrast to fructokinase activities, which were highest in the proximal small intestine. There is no apparent explanation for this difference in enzyme distribution along the gut. The distribution of fructokinase is compatible with a specific induction mechanism for the utilization of dietary fructose by jejunal epithelium of rats on high-fructose or high-sucrose diets.

Data in Table 4 show that intestinal fructokinase activity increased significantly on high-fructose or high-sucrose diets (Table 4). Similar results were obtained by Stiefel et al. (16), although their increases were of greater magnitude. Their data also indicated a greater stimulation of activity by high-fructose as compared to high-sucrose diets. We found no significant difference between a high-sucrose or high-fructose diet. We also demonstrated by DEAE-cellulose chromatography (Table 5) that the increase in fructokinase activity appears to be specific for the isolated enzyme. The data on intestinal fructokinase are similar to that obtained with liver fructokinase by Adelman et al. (1). They, too, found that noncarbohydrate diets maintained a low liver fructokinase level similar to fasting while a high-fructose diet markedly increased this activity.

In conclusion, the data presented here do not support an adaptive response of intestinal hexokinase to diet, if one infers from that term control of enzyme synthesis. A significant change in intestinal hexokinase activity in response to dietary glucose was apparent only when compared to a noncarbohydrate diet. The magnitude of this change was relatively small and appeared to be secondary to a local cellular increase in glucose concentration in the actively absorbing cell. In addition, there was no evidence for the presence of an intestinal glucokinase response to diet. Thus, it is clear that more extensive studies are needed on the molecular mechanism responsible for changes in intestinal glycolytic enzyme levels before one can attribute any etiologic role to a maladaptive response of these enzymes in diseased states.

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Hexokinase isoenzymes in liver and adipose tissue of man and dog.
